

Polymeric derivatives of plant growth regulators: synthesis and properties

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Received 6 February 1993; accepted 7 July 1993

Key words: polymeric plant growth regulators, synthesis, controlled release, hydrolysis, biological activity

Abstract

The polymeric formulations of plant growth regulators (PGRs) are high molecular weight systems in which the PGR unit is attached to the polymeric chain by a hydrolysable chemical bond. These polymeric derivatives (esters, ethers, or else) of PGRs are characterised by the ability to release the active compound (PGR) from their solutions (mainly aqueous) in certain conditions. The release of the PGR can be controlled by external factors (pH, temperature, enzymes, solution concentration), and inherent properties of the whole macrosystem chemical structure, such as the type of the hydrolysable bond between PGR unit and the main polymeric chain, the structure of the polymer chain (e.g. molecular weight, level of hydrophilicity, and the content of hydrophobic groups, macromolecular conformation in solution etc.). These controlled (slow) release PGRs display certain advantages over conventional PGR formulations due to their prolonged action, improved efficiency (e.g. wide range of effective concentrations) greater safety to non-target organisms and the applicators. In addition the ability of altering the solubility level and modifying the application form is of considerable interest. The biological activity efficiency of polymeric PGRs has been documented and the relation of this efficiency to the PGR unit hydrolytic release ability has been mentioned. Slow release polymeric PGRs are considered to solve certain problems in agriculture.

Abbreviations: PGR = plant growth regulator; C(S)RF = controlled (slow) release form; PD = polymeric derivative; ACC = 1-amino-cyclopropane-1-carboxylic acid; NAA = 1-naphthylacetic acid; 2,4-D = 2,4-dichlorophenoxyacetic acid; IAA = indole-3-acetic acid; BAP = N⁶-benzylaminopurine; ABA = abscisic acid; GA = gibberellin; LMW = low molecular weight; HMW = high molecular weight.

1. Introduction

Phytohormones (including the most extensively studied auxins, gibberellins, cytokinins, abscisic acid, ethylene and its precursor 1-aminocyclopropane-1-carboxylic acid) as well as other substances, (particularly fusicoccin, brassinosteroids, aliphatic polyamines and plant cell wall oligosaccharides), are involved in the endogenous regulation of plant growth [15, 26, 58, 73]. The exogenous regulation of plant growth include phytohormones and other natural substances (e.g. vitamins, nucleotides) as well as many of their synthetic analogues and other synthetic compounds. Over time, these plant growth regulators PGRs have gained some

practical relevance [e.g. 25, 58]. However wide spread use of these PGRs has been limited by a number of factors [e.g. 1, 8, 12, 41, 75, 82]. As a rule, the most active compounds in terms of their biological effects are characterized by a narrow region of effective concentrations and doses. If these concentrations or applied doses are exceeded, then plants rather than being stimulated may be inhibited even to the point of death (herbicidal effect). In addition, many PGRs, especially those of natural origin or their synthetic analogues, are biodegradable. While other are easily washed away or are volatile or toxic to organisms and the environment.

For the last twenty years, increasing attention has

been focused on systems based on various preparation forms which are able to release a bioactive compound at a certain rate. The rate obtained is dependent mainly on the general preparation of the system and in particular, the structure of its ingredients. The theory of controlled release and its applications in medicine, biotechnology and agriculture has been well documented [e.g. 12, 46, 48]. A great number of different systems for controlled release have been investigated, primarily based on two mechanisms of release, i.e. diffusion and cleavage of chemical bonds. Controlled release systems may be prepared in different forms. The active ingredients may be modified with various polymeric materials to give vesicles, micro and macrocapsules, nanoparticles, sheets, gels, dusts, fibers, filaments, membrane coating systems etc.

Controlled release systems for agriculture based on the diffusion mechanism [e.g. 4–9, 82, 46] and also on hydrolysis of chemical bonds between the herbicide (or biocide) unit and the polymeric matrix (carrier) [e.g. 4, 7, 22, 41, 50, 63] have been prepared. These principles have been also utilized [8, 9, 54, 70, 78] for the modification of PGRs. The polymeric derivatives (PD) of the latter have been referred to, in some reports, as phytoactive polymers [68, 71, 72, 77, 81].

2. Phytoactive polymers: slow release PGR formulations

In general, the macromolecule of a phytoactive polymer is a high molecular weight (HMW) system in which the bioactive compound unit (e.g. PGR) is attached to the polymeric chain by a hydrolysable chemical bond. The plant growth regulator units may either be introduced directly onto the chain, attached to the terminal linkages of the polymeric chain, or attached to the side groups of the main polymeric chain [8, 41, 54, 76]. The latter method of linking the regulator unit to a polymeric chain has been shown to be the most promising since in this case both the content of bioactive compound in the polymer and also the properties of the overall polymeric system may be regulated during synthesis. The regulator units may be either directly attached to the side polymer linkage or through a spacer. The hydrolysable bond between polymeric chain and PGR units may be ionic, covalent, or of coordination

character. The functional moieties most often used for covalent bonding are esters, amides, urea, carbamates etc. The type of hydrolysable bond depends upon the reactive groups that the particular low molecular weight (LMW) bioactive compound structure contains. In addition to linkages with the LMW regulator units, the phytoactive polymers may contain other linkages in order to provide the polymeric system with the appropriate hydrophilicity (e.g. hydroxylic groups, amidic groups, pyrrolidonic fragments, ionogenic groups). For some applications, it may be necessary to decrease the water solubility of the PGR and this can be achieved by its incorporation into an appropriate polymer matrix.

Different methods of phytoactive polymer synthesis have been reported. However, most often homo- and co-polymerization of the unsaturated derivatives of LMW regulators or reaction of the LMW regulators with reactionable polymeric carriers were used [5, 77, 81]. It should be mentioned that methods of synthesis of phytoactive polymers are similar to those of other biologically active HMW compounds e.g. immobilized pharmaceuticals [60, 69] and biocides [1, 4, 5, 7, 50, 63, 69]. In addition, the investigation of synthetic methods for preparation of phytoactive polymers and those of PD of certain herbicides have co-developed. A biological active compound (e.g. pesticide), according to the concentration applied, may display either a stimulating or an inhibiting effect. [4, 7, 63, 70, 76]. The choice of a method for the synthesis of a phytoactive polymer depends upon the accessibility of the LMW bioactive compound, its ability to produce activated derivatives and its stability in chemical processes. Even in cases where the same LMW bioactive compound has been attached to the polymeric system, the properties of the prepared phytoactive polymers depend on specificities of the polymeric system chemical structure.

3. Synthesis of phytoactive polymers: polymeric derivatives of PGRs

3.1 Polymeric derivatives of auxins

Various aryl- and aryloxyalkanecarboxylic acids possess auxin-like activity (e.g. the ability to stimulate roots and callus formation). A notable number

of PD of auxins have been documented. In most cases haloidaryloxyalkanecarboxylic acids were used as the LMW bioactive compound. Some of these acids, in addition to regulating plant growth activity, may display biocidal and herbicidal activity [5, 76]. Early studies on the synthesis of PD of phytohormonal auxins, such as 3-indolylacetic acid, heteroauxin, their synthetic analogues 3-indolylpropionic acid, 3-indolylbutyric acid and 1-naphthylacetic acid were, however, rather poor. When abilities for the preparation of polymeric systems containing auxin units were examined, it was found that the unsaturated esters of aryloxyalkanecarboxylic acid showed good ability for homo- and copolymerization [61]. The unsaturated derivatives of the arylalkanecarboxylic acids were characterized by a lower ability to homo-polymerization [33, 34]. Despite this, examples of co-polymerization reactions involving unsaturated derivatives of 1-naphthylacetic and 3-indolylacetic acids as co-monomers were referred to [51, 53].

A considerable number of investigations were devoted to the linking of the auxinic class carboxylic acids or their derivatives (chloroanhydrides or salts) onto natural or synthetic polymeric carriers containing various reactive groups. In cases where the preparation of the polymeric esters was based on polymer carriers containing hydroxy groups, the chloroanhydrides of auxins usually were involved in the reaction. The use of natural polymers containing hydroxy groups (e.g. cellulose, starch, alginic acid, dextran, chitin, chitosan, and also lignin and various wood waste) as carriers for the immobilization of haloidaryloxy-carboxylic acids [5, 7] and 1-naphthylacetic acid has been documented [10]. The reactions of 1-naphthylacetic and 3-indolylacetic acid chloroanhydrides with synthetic polymers containing hydroxy groups, such as, polyvinylalcohol and poly(N-hydroxymethylmaleimide) [30, 33] and also of 2,4-dichlorophenoxyacetic and 2-methyl-4-chlorophenoxyacetic acid chloroanhydrides with gels of 2-hydroxyethylmethacrylate, acrylamide and methylene-bis-acrylamide copolymers were also described [23].

The nonactivated carboxylic acids from the class of auxins, in reaction with polymers containing hydroxy groups rarely were involved. One example, however, was the esterification of the polyvinylalcohol with 2,4-dichlorophenoxyacetic acid,

which was achieved in melting conditions. When polymeric carriers containing activated haloid-alkyl groups (e.g. chloromethylated polystyrene, poly-N-chloromethylmaleimide, polyvinylchloromethylketone or co-polymers of vinyl-2-chloroethyl esters [17]) were used, then the LMW compounds were involved in the synthesis in non-activated form, such as salts of acids or as the acids themselves. In this case, the reaction was carried out in the presence of hydrogen chloride acceptors [24, 33].

A broad number of PD of auxins (e.g. haloidaryloxyalkanecarboxylic, naphthylacetic, phenylacetic and indoleacetic) have been prepared by their reaction of the LMW acids with polymeric epoxides (e.g. polyglycidylmethacrylate) [40, 43]. In this instance, the acids have been directly introduced in the reaction [31, 32, 38, 39, 65]. The PD of the auxins which have been prepared in the above cases displayed a different ability to release the biologically active compound [68].

3.2 Polymeric derivatives of gibberellic acid

The most widely studied representative of the gibberellins is the gibberellic acid (Gibberellin A₃, GA). Some processes for the preparation of PD of GA already have been described. The ability to use the bonded to agarose GA as immobilized ligand for the preparation of affinity sorbents has been mentioned [44]. At the same time, PD of GA suitable for use as PGRs were prepared by reaction of GA or of its potassium salt with polymer carriers containing epoxide or chloroalkyl groups. In these cases, products both soluble and insoluble in water were formed [56]. By reaction of the GA with poly-2-methyl-5-vinyl-1-pyridinemethylenepentamethyl disiloxane, polymeric salts showing low biological activity were prepared [27].

3.3 Polymers with cytokinin activity

PD of cytokinins which induce, in particular, the process of cell division are described in a series of scientific papers. The main representatives of these polymers are derivatives of adenine, one of the nucleic bases. The immobilization of a series of 6-N-substituted adenins on polysaccharides (cellulose and starch) has been achieved by previous treatment of the polysaccharides with phosgene in

order to introduce in them the reactive chlorformate groups [12, 13]. PD of cytokinins also have been obtained by attaching 6-N-substituted adenins to polymeric carriers containing isothiocyanate groups. In this case, the regulator units were linked to a polymeric chain by a thiourea spacer. Among other polymers with cytokinic activity, one should mention the PD of 6-[N-(2,3-dihydroxypropyl)amino]purine, of 5'-adenosinephosphates and N⁹-(2,3-dihydroxypropyl)aminopurine. The first of the above polymers was prepared by reaction of the LMW cytokinin with a polymer containing acetale groups, while the second and the third polymers were prepared by reaction of 5'-adenosinephosphates and adenine with polyglycidylmethacrylate [35, 36, 42, 66]. The substitution reaction of the multifunctional molecule of adenosinephosphates when treated with the oxiranic cycle (of polyglycidylmethacrylate) proceeded mainly through the hydroxy group of the adenosine phosphonic acid residue. The above was confirmed by analysis of the products of a model reaction between adenosin-5-phosphate and glycidol.

3.4 Polymeric derivatives of ethylene generating compounds

Phytohormonal ethylene plays a very important role in plant life. The possibility of its immobilization in polymeric systems in order to obtain growth regulating systems is indicated in the literature [9]. 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor compound in the metabolic pathway of ethylene synthesis in plants [84, 85], during exogenous application in plants displayed biological effect similar to that of ethylene [64, 69]. The synthesis of certain PD of ACC possessing biological activity has been reported [64]. These HMW compounds have been prepared by various methods, such as, the co-polymerization of N-methacryloylate derivative of the acid with N-vinylpyrrolidone, the reaction of 1-isocyanatocyclopropyl-1-carboxylic acid methyl ester with polyvinylalcohol and the reaction of 1-aminocyclopropane-1-carboxylic acid with copolymers of maleic anhydride [64, 77]. A polymeric salt of another ethylene producent the 2-chloroethyl-phosphonic acid was obtained by reaction of the last with polymer containing basic groups [68].

3.5 Polymeric derivatives of other regulators

Some attempts to prepare polyvinyl esters of succinic and sorbinic acids have been described [29, 30]. The above acids in their LMW form possess certain plant growth regulating activity. The polymeric esters of the vitamin 3-pyridinecarbonic (nicotinic) acid also have been found to have notably high biological activity. These polymeric PGRs have been prepared by the acylation of polyvinylalcohol with the chloroanhydride of the nicotinic acid [35, 37] or by the reaction of the acid itself with polyglycidylmethacrylate. Different authors have referred to the preparation of biologically active PD of substituted acetylene, copolymers of bis-trialkylstannyl maleinates and polymer based on N,N-dimethylhydrazide of the succinic acid [16]. The preparation of the polymeric salt of polyacrylate acid and the N-oxide alkaloid of lutidine has been achieved by photoinitiated polymerization of the corresponding monomers. The biological activity of some synthesized polymeric forms of fusicoccin derivatives has also been mentioned. It should be mentioned that references related to the synthesis of PD of the abscisic acid or its derivatives that display biological activity have not been found in the scientific literature. Despite that, examples of abscisic acid immobilization on polymers in order to obtain affinic sorbents are known [47].

4. Properties of the polymeric derivatives of PGRs

4.1 Solubility

Depending on their chemical structure, the phytoactive polymers are soluble or insoluble in water, or organic solvents solid compounds (powders). The modification of the LMW biologically active compound to its polymeric form provides the ability of altering the solubility level. For example, by increasing the number of the hydrophilic groups, which are introduced in the polymeric chain and also the level of their hydrophilicity, water soluble forms of the PGRs, even for such hydrophobic compounds as the fusicoccin, may be obtained. The water solubility of the final polymeric products with plant growth regulating activity and controlled release of the bioactive LMW

compound is very important in terms of application abilities, particularly when traditional technologies are used. In addition, the lack of solubility of the PGRs or their PD makes their uniform distribution on the treated objects impossible since very low doses of the biologically active compounds or their modified forms are usually used.

In relation to the above facts, the application of controlled release PGR preparations, in cases where the release of the bioactive compound, was based on diffusion mechanisms evoked certain complexities. Such preparations containing adenins, gibberellic acid, 2,4-dichlorophenoxyacetic acid, benzoic acid, 1-naphthylacetic acid have been reported. In the above formulations, the active compounds have been included inside micro-porous cellulose fibers or dissolved in polymeric matrices [9]. By way of contrast, in some particular cases, when the LMW bioactive compound is easily washed away, its incorporation into the polymeric carrier may be necessary for certain applications in order to decrease the water solubility of the final product.

4.2. Hydrolysis ability

A very important characteristic of the phytoactive polymers is their ability in solutions (mainly aqueous) gradually to release the LMW bioactive compound by cleavage of the bond between the bioactive substance units and the polymeric chain. In a few reports the plant regulating polymers which do not contain immobilized LMW regulators were found. Nevertheless it is still not clear in these cases, if the appearance of the biological effect is due to the polymers themselves, to the presence of little amounts of LMW admixtures within them, or to any other reasons. It is important to note, that the relative role of chemical and enzymatic hydrolysis in the phytoactive polymer function still has not been studied well. Also, investigations concerning the penetration of the released bioactive LMW compound in the plant and the way of its involvement in the metabolic processes of plants have not yet been conducted. Hence, a noticeable number of investigations have been devoted to hydrolysis studies of the polymeric PGRs in model conditions [e.g. 21, 79, 80]. From the data already obtained, conclusions may be drawn about the substantial influence of the

final polymeric system chemical structure to the release ability of the LMW bioactive compound from the polymer.

It may be stated that the main factors which influence the release rate of the LMW bioactive compound are: 1) the type of spacer and hydrolysable group between polymeric chain and immobilized bioactive ligand, 2) the level of overall system polarity and its solubility in water, 3) the type of lyophilic groups, 4) the level of hydrophobic nature of the bioactive compound unit, 5) the molecular mass of the polymer and 6) the configuration of the polymer macromolecular structure in solution. For example, in the case of the PD of ACC when the ACC unit was attached to the polymeric carrier by various types of hydrolysable groups, it was found that the hydrolysis rate was strongly dependent on the type of hydrolysable group. In particular, increase of hydrolysis rate was observed by changing the amide or imide group to a carbamate one [77]. The hydrolysis rate of phytoactive polymers was elevated by increasing the polarity of the side spacer (linkage). This was proved in a series of products, (e.g., for the water soluble polymeric esters of 1-naphthylacetic acid [80], the gel copolymers of acrylamide and oligooxyethylenemethacrylate esters of 2,4-dichlorophenoxyacetic and 2-methyl-4-chlorophenoxy acetic acids [61], the water soluble copolymers of acrylamide and oxybutylacrylate, the oxyethylacrylate and oxyethylmethacrylate esters of 1-naphthylacetic acid, the oxyethylacrylate ester of 3-indolylacetic acid [52], and also for some other LMW compounds [67].

Hydrolysis studies of water soluble polymeric esters of 1-naphthylacetic acid containing acrylamide hydrophilic groups showed that the hydrolysis rate in alkaline conditions is much higher than that in strongly acidic ones. In smooth acidic and neutral media hydrolysis proceeds at an insignificant rate [80]. As was demonstrated for polymeric esters of 1-naphthylacetic acid, the molecular mass of the phytoactive polymer substantially affected the hydrolysis rate of the polymers in homogenic media, especially if the molecular mass reached values of the range 10,000 to 100,000 [52, 80]. A similar influence, i.e. a slow down of the hydrolysis rate by increasing the molecular mass value also was noted in the hydrolysis studies of the polymeric derivatives of biologically

active carbamates. On the other hand, by increasing the content of the hydrophobic regulator residues in the polymeric system, a slow down of the hydrolysis rate was shown as well [52, 80]. On the other hand, biological *in vitro* studies have been conducted to evaluate the influence of enzymes on the hydrolysis of the PD of biological active carbamates and the PD of ACC. It was shown that enzymes contributed positively to the release of the LMW PGR from the polymer [64]. This evidence seems to suggest that enzymatic processes may play a certain, perhaps mayor role, on the hydrolysis of the polymeric PGRs. Hence experimental data concerning such processes in nature and involving polymeric PGRs have not been found in the literature.

4.3 Biological activity

The already existing data allows consideration of some substantial differences in the biological activity between the LMW regulators and their corresponding polymeric forms. Particularly, in most of the investigations, a shift of the stimulating doses and/or concentrations (of the polymers or polymer solutions respectively) towards higher values and an extension in width of the values of the stimulating region has been exhibited. In several experiments in which very high polymer concentrations were used no inhibitory effect were reported on plant material (e.g. whole plants, explants, seeds, etc). Investigations on the biological activity of the phytoactive polymers have been described in original articles, patents and in conference proceedings [e.g. 2, 10, 14, 18–20, 59, 75, 77, 81].

Comparative studies on the biological effect between the LMW regulators and their corresponding PD on various plant materials has been performed in several cases. Cytokinins were tested on callus growth stimulation of soybean [12, 13], and stimulation of the nitrate reductase activity and amaranthine synthesis in amaranthus embryos [68, 75]. Auxins and their PD were tested for the stimulation of respiration intensity in tobacco tissue culture and growth of roots of various plants. Gibberellins and their PD were used in biotests for growth of lettuce seed hypocotyles and of wheat coleoptile segment elongation [56].

The positive influence of the chemical structure of phytoactive polymers on their biological activity has

been documented in experimental data [e.g. 59, 77]. It has been particularly demonstrated that a certain correlation exists between the hydrolysis ability of the phytoactive polymers and the level of their bioactivity. No hydrolysis, was always associated with lack of response. If the polymer molecular mass, or the hydrophobic linkages of the polymers are increased the hydrolysis rate is decreased. In this case, as a rule, of greater concentrations are needed to get the desirable stimulating effects. On the contrary, by introducing more hydrophilic substitutes in the polymer [69] the stimulating concentrations needed were of lower values.

A series of biotests were devoted to the influence of the phytoactive polymers on various vital peculiarities of the plants. Furthermore, the effect of cytokinins and their PD was studied on the reflective properties of leaves and also on water absorbance property of winter wheat. Also the free-radical oxidation of lipids [28] and other physiological processes of some cultures in conditions of water-stress [19, 20] were studied. The influence of phytoactive polymer application to the structure of the polar lipids of the chloroplast membranes and their ultra-structure [14, 19] and to the content of endogenous cytokinins and adenosinephosphates in the leaves of winter wheat and potatoes has been studied. Other biological studies concerning the influence of various phytoactive polymers on the content of chlorophyll in the leaves of wheat [55], on the mesostructure of potatoe leaves [83] have also been performed. It has been also reported that PD of PGRs increased the tolerance of maize in high salinity soils, the tolerance of plants in conditions of low temperatures [14, 74] and the tolerance of sweet peas plant against the damaging action of metals (iron, manganese, chromium) [11].

Phytoactive polymers with auxin-like activity affected the accumulation of mineral substances in the leaves of basma and henna plants and the germination and growing power of the tomato seeds and sprouts of cereals in soils high in chloride salts [2, 3]. Reports concerning the influence of PD of auxins and other PGRs on the functional protein content of henna leaves, on the formation changes in root mitochondria of rose cuttings [20], and on the regulation of the physiological processes in conditions of water-stress [19, 20] have also been published. In addition the positive effect

of polymeric auxins on the growth of maize seedlings and plants [69], on the increase of seed quality and crop yield of sugarbeet plants [18], and on the increase in productivity of soil producing crops as well as citrus crops [69] has been documented.

Data on biological activity of other types of phytoactive polymers are comparatively limited. The treatment of corn seeds with the polyvinyl ester of nicotinic acid has resulted in notable growth stimulation [65, 71]. The application of the polymeric derivative of 2-chloroethylphosphonic acid to cereals has produced a remarkable retardant effect. The biological activity of polymeric esters of GA [56] and of the biological active carbamates has been documented in standard bio-tests. The application of the polymeric form of fusaric acid on winter wheat has been shown to improve the wheat frost-resistant properties [57]. It has been demonstrated that PD of ACC produce a synergic stimulation on the nitrate reductase activity of amaranthus embryos, when applied together with cytokinins (e.g. BAP) higher than that of LMW ACC [64]. The influence of the polymer structure to other biological activities, such as callus formation on leaf-segments of tobacco and grapevine genotypes [59] has been also examined.

5. Conclusions

It may be concluded that even though investigations into the area of phytoactive polymers are relatively recent, a great number of representatives from this new generation group of biologically active HMW compounds now have been synthesized. On the basis of the experimental data, concerning the effect of the phytoactive polymers on plant life, it may also be concluded that application of these polymers to agronomy is very promising. It is clear that further investigations into new methods of polymeric PGR preparation, and the relation between the properties of polymeric PGRs, their structure, and their biological activity should be promoted.

Acknowledgement

We wish to thank Thanasis K. Alegakis (B.S.) for typing and preparing the manuscript. Finan-

cial support from University of Crete is greatly appreciated.

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